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## Pathogenesis of feline enteric coronavirus infection

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Fifty-one specific pathogen-free (SPF) cats 10 weeks to 13 years of age were infected with a cat-to-cat fecal–oral passed strain of feline enteric coronavirus (FECV). Clinical signs ranged from unapparent to a mild and self-limiting diarrhea. Twenty-nine of these cats were FECV naïve before infection and followed sequentially for fecal virus shedding and antibody responses over a period of 8–48 months. Fecal shedding, as determined by real-time polymerase chain reaction (RT-PCR) from rectal swabs, appeared within a week and was significantly higher in kittens than older cats. FECV shedding remained at high levels for 2–10 months before eventually evolving into one of three excretion patterns. Eleven cats shed the virus persistently at varying levels over an observation period of 9–24 months. Eleven cats appeared to have periods of virus shedding interlaced with periods of non-shedding (intermittent or recurrent shedders), and seven cats ceased shedding after 5–19 months (average 12 months). There was no change in the patterns of virus shedding among cats that were excreting FECV at the time of a secondary challenge exposure. Four cats, which had ceased shedding, re-manifested a primary type infection when secondarily infected. Cats with higher feline coronavirus (FCoV) antibody titers were significantly more likely to shed virus, while cats with lower titers were significantly less likely to be shedding. Twenty-two kittens born to experimentally infected project queens began shedding virus spontaneously, but never before 9–10 weeks of age. Natural kittenhood infections appeared to be low grade and abortive. However, a characteristic primary type infection occurred following experimental infection with FECV at 12–15 weeks of age. Pregnancy, parturition and lactation had no influence on fecal shedding by queens. Methylprednisolone acetate treatment did not induce non-shedders to shed and shedders to increase shedding.

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**F**eline enteric coronavirus (FECV) is a ubiquitous, worldwide, intestinal virus of cats (Pedersen et al 1981a, 2004). The name feline coronavirus (FCoV) has been applied somewhat interchangeably to FECV. Technically, FCoV includes all strains (numerous), serotypes (types I and II) and biotypes (enteric or infectious peritonitis viruses) of the genus. Several strains of FECV have been studied by experimental fecal-oral infection with cat-to-cat passed virus. The original FECV strain was designated FECV-University of California, Davis (UCD) (Pedersen et al 1981a) and a second isolate FECV-Rogers

and Morris (RM) (Hickman et al 1995). Both of these strains belong to serotype I, possessing a feline- rather than canine-coronavirus spike protein. Several additional FECV strains have been studied in the field using polymerase chain reaction (PCR) (Foley et al 1997b, Benetka et al 2006). FECV is tropic for the mature apical epithelium of the intestinal villi (Pedersen et al 1981a) and both type I and II serotypes use species- and probably type-specific (Dye et al 2007) variants of aminopeptidase-N as a receptor (Tresnan et al 1996, Tusell et al 2007). FECV infection is usually unapparent or manifested by a transient gastroenteritis (Hayashi et al 1982, Pedersen et al 1981a, Mochizuki et al 1999); it is rarely fatal when in its native biotype (Kipar et al 1998).

The importance of FECV as a primary intestinal pathogen is minimal. However, FECV commonly

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mutates in vivo and at least one mutant form (ie, biotype) causes a highly fatal disease known as feline infectious peritonitis (FIP) (Poland et al 1996, Vennema et al 1998). The precise nature of the mutation that causes this change in virulence has been variably ascribed to differences in the spike protein (Rottier et al 2005) or to non-synonymous or deletion mutations in the 3c (small envelope) gene (Vennema et al 1998). The incidence of the enteric → FIP biotype mutation following FECV infection is unknown but may be as high as 20% (Poland et al 1996) and is more likely to manifest clinically in kittens (Foley et al 1997a) or immunocompromised cats (Poland et al 1996). FIP virus (FIPV) differs from its strictly intestinal tropic FECV parent in its affinity for macrophages (Pedersen 1987, Stoddart and Scott 1989). This altered tropism allows the virus to become a systemic pathogen of macrophages, and the resultant disease involves a complex interaction between host cellular and humoral immunity and infected macrophages (Pedersen and Boyle 1980 Pedersen 1987).

Although there have been numerous studies of FIPVs, studies of FECVs have been surprisingly few. Experimentation with FECV has been hampered by its lack of growth in tissue culture. Therefore, infection studies have often relied on extracts of feces from cats infected with cat-to-cat passed virus (Pedersen et al 1981a, Poland et al 1996). Although one report suggests that a cultured strain of FCoV, WSU-79-1683, is a prototypic FECV (Pedersen et al 1984b), this author now believes it to be a tissue culture attenuated recombinant of canine and feline coronavirus. This is given support by the complex patterns of recombination that have been described for WSU-79-1146 (a highly virulent FIPV) and WSU-79-1683, which were both isolated from the same laboratory at the same time (Herrewegh et al 1998). WSU-79-1683 also lacks the 7b gene, which is intact in cat passed FECVs (Herrewegh et al 1995). Therefore, studies of FECV should use biotype confirmed fecal passaged virus until a proper FECV is adapted to tissue culture.

The present study was designed initially to prove that resistance and susceptibility to FECV infection were under genetic control, just as genetics appears to play an important role in FIPV resistance (Foley et al 1997a). Young cats were infected with the RM strain of FECV and their patterns of fecal virus shedding quantified over extended periods of time by periodic sampling. Cats that stopped shedding the virus after

8–12 months were than bred to cats with a similar profile, and cats that appeared to be long-term shedders were bred to chronic shedders. Their kittens were then infected with FECV at 10–23 weeks of age and the cycle continued. The goal was to create two bloodlines, one resistant and one susceptible. Once this was accomplished, the genetic basis for resistance/susceptibility was to be determined. After more than 3 years, it became apparent that FECV resistance and susceptibility may not be definable by simple Mendelian genetics. Therefore, a decision was made to concentrate on what was learned about FECV pathogenesis.

## Methods

### *Experimental animals*

Twenty-nine FECV naïve cats, ranging from kittens to aged animals, were obtained from the specific pathogen-free (SPF) breeding colony of the Feline Nutrition Laboratory, UC Davis. Cats were housed in the feline research facilities of the Center for Companion Animal Health (CCAH), UC Davis. Care was provided by staff of the CCAH under the supervision of the Center for Laboratory Animal Services, UC Davis. Studies were done under United States Department of Agriculture required Institutional Animal Care and Use Committee approved protocols. Males and females were not neutered for this study. Select animals were chosen for breeding during the course of the study and 22 kittens produced from these mating's added to the study over time.

### *Experimental infection*

Cats were infected with 0.5 ml orally of a fecal extract (Poland et al 1996) of the RM strain of FECV (Hickman et al 1995). The initial group of cats was infected several days after acquisition, while kittens reared during the study were infected at 12–15 weeks of age and observed for signs of acute or chronic disease. Cats were housed in open rooms, with no more than five animals per room. These groups remained relatively stable, except when toms or queens were transferred for breeding or queens isolated for birthing and kitten rearing. Reasonable precautions were taken to limit spread of contaminated litter by caretakers; disposable coveralls, boots, foot baths, hand washing, gloves were used.

### Quantitation of FECV shedding

FCoV RNA was quantified using purification procedures and specific primers reported by Gut et al (1999). Feces were collected by inserting standard cotton tipped swabs into the rectum prior to infection and at 1 week intervals for at least 2 months, and then at 1–2 month intervals thereafter. RNA was isolated from the swabs (van der Hoek et al 1995). Five microliters of the purified RNA was added to 7  $\mu$ l of PCR mixture containing 6  $\mu$ l of TaqMan One Step RT-Master Mix (Applied Biosystems, Foster City, CA), 0.31  $\mu$ l of MuLV/RNase Inhibitor, 0.24  $\mu$ l each of forward and reverse primers, and 0.10  $\mu$ l of RNase-free water. The 12- $\mu$ l reaction went through a reverse transcriptase step for 30 min at 48°C and AmpliTaq Gold (Applied Biosystems, Foster City, CA) activation for 10 min at 95°C. The samples were put through 40 cycles of 95°C for 15 s and 60°C for 60 s for RNA amplification. PCR was performed using Applied Biosystems (Foster City, CA) 7300 Real-time polymerase chain reaction (RT-PCR) System and 7300 System Software. The positive/negative cutoff of the assay was around 75–100 RNA transcripts/swab. Therefore, swabs that were negative at  $1 \times \log 10$  were considered negative. The number of RNA transcripts per swab was considered equal to the number of viral particle (Gut et al 1999), given that each FECV particle contains only one RNA transcript. There was no evidence for fecal inhibitors of the RT-PCR assay used in this study; SPF cat fecal samples were always negative, but became rapidly and progressively positive after experimental infection. Therefore, internal DNA (Monteiro et al 1997) or RNA (Escobar-Herrera et al 2006) fragment controls were not employed.

### FCoV antibody tests

Serum antibody titers to FECV were undertaken with an indirect fluorescent antibody (IFA) procedure (Pedersen 1976) using FIPV-UCD1 infected Fcwf-4 cells (Pedersen et al 1981b). Cells were grown in 12-well Teflon coated microscopic slides and infected with FIPV-UCD1 tissue culture fluid when three-quarters confluent. Slides were harvested after 24–48 h and fixed in absolute acetone. Each serum was tested at 1:5, 1:25, 1:100, 1:400 and 1:1600 dilutions in Hank's buffered saline solution. Serum was allowed to react for 1 h, slides washed, and a 1:50 dilution of rabbit anti-cat IgG (Antibodies Incorporated, Davis, CA 95616) was over layered for 1 h. Slides were then washed, stained with dilute Evan's blue dye, and cover slips mounted with 1:1 glycerin:saline.

Slides were read on an indirect fluorescent microscope and the titer listed as the last dilution of serum that still produced noticeable fluorescence.

### Statistical analysis

Data was recorded on Excel spread sheets (Microsoft Office 2003, Microsoft, Redmond, WA 98004), and statistical analyses, when indicated, undertaken with JMP Statistical Discovery Software (SAS, Cary, NC 27513) ([www.jmp.com/software/](http://www.jmp.com/software/)). Significance ( $P \leq 0.05$ ) was determined by the program's Student *t*-test.

## Results

### Outcome of primary infection

Thirty-three cats were infected with FECV and followed sequentially for fecal virus shedding over a period of 14–48 months (Table 1). Twenty-nine cats were FECV naïve at the start of the study. Four of these cats (A01–A04) were born during the course of the study to project queens and, therefore, not FECV naïve, but were virus negative at the time of primary infection. Fecal shedding rose within a week and remained at consistently high levels of  $10^{12}$ – $10^{16}$  particles/swab for 2–10 months (Figs 1–3; Table 1). Peak virus levels tended to drop to levels of  $10^6$ – $10^9$  particles per swab in the secondary stage of infection that followed (Figs 1–3).

Three different patterns of virus shedding were noted in the secondary infection stage. Eleven cats shed the virus continuously at greatly varying levels over an observation period of 14–24 months (persistent infection) (Table 1; Fig 1). Twelve cats had brief periods of recovery, interlaced with periods of virus shedding (intermittent or recurrent shedders) (Table 1; Fig 2), and 10 cats ceased shedding at 7–18 months (average 12.3 months) (Table 1; Fig 3). Three representative cats were graphed for each of the three infection outcomes (Figs 1–3). None of the cats developed FIP.

### Outcome of secondary infection

Nineteen cats were used for this study and divided into two groups of four and 15 based on their virus shedding patterns prior to reinfection. The four cats that had low or non-measurable virus shedding at the time of secondary exposure were clearly reinfected. Fecal shedding for one of these cats is illustrated in Fig 4. Figure 5 shows the mean virus

**Table 1.** Description of 33 cats used to study patterns of fecal FECV shedding following primary infection and in the study on the effect of methylprednisolone acetate induced stress in 18 of these animals

| Cat number | Gender/age (months) | Observation period (months) | Duration of primary infection (months) | Outcome of infection | Time to recovery (months) |
|------------|---------------------|-----------------------------|--|----------------------|---------------------------|
| 94309      | F/151               | 16                          | 4                                      | Persistent           | NA*                       |
| 94529      | F/154               | 16                          | 3                                      | Recurrent            | NA                        |
| 98462      | F/108               | 13                          | 3                                      | Recurrent            | NA                        |
| 99402      | F/96                | 12                          | 6                                      | Recurrent            | NA                        |
| 00417      | F/96                | 14                          | 4                                      | Recurrent            | NA                        |
| 01282      | F/33                | 13                          | 2                                      | Recurrent            | NA                        |
| 02136†     | F/59                | 48                          | 5                                      | Recurrent            | NA                        |
| 04096      | F/51                | 10                          | 4                                      | Persistent           | NA                        |
| 04139      | F/52                | 13                          | 10                                     | Recurrent            | NA                        |
| 04140      | F/52                | 13                          | 8                                      | Recurrent            | NA                        |
| 04141      | F/52                | 10                          | 2                                      | Persistent           | NA                        |
| 04144      | F/52                | 9                           | 2                                      | Persistent           | NA                        |
| 04146      | F/52                | 9                           | 3                                      | Persistent           | NA                        |
| 04161      | M/51                | 9                           | 2                                      | Persistent           | NA                        |
| 04224      | M/50                | 9                           | 4                                      | Persistent           | NA                        |
| 04225      | M/50                | 12                          | 5                                      | Persistent           | NA                        |
| 98272†     | M/90                | 24                          | 4                                      | Persistent           | NA                        |
| 04099‡     | M/51                | 36                          | 4                                      | Recurrent            | NA                        |
| 05243†     | F/52                | 24                          | 3                                      | Recurrent            | NA                        |
| 05244‡     | M/52                | 24                          | 3                                      | Recurrent            | NA                        |
| 05246†     | F/52                | 23                          | 5                                      | Recovered            | 19                        |
| 05249‡     | F/52                | 23                          | 5                                      | Recovered            | 18                        |
| 05325‡     | M/52                | 23                          | 5                                      | Persistent           | NA                        |
| 05326†     | M/52                | 23                          | 5                                      | Persistent           | NA                        |
| 06028†     | M/52                | 14                          | 2                                      | Recovered            | 15                        |
| 06029‡     | F/53                | 14                          | 2                                      | Recovered            | 15                        |
| 06032†     | F/53                | 14                          | 4                                      | Recovered            | 12                        |
| 06033‡     | F/53                | 14                          | 2                                      | Recovered            | 12                        |
| 06034†     | M/53                | 14                          | 3                                      | Recovered            | 15                        |
| A01‡       | M/2                 | 13                          | 4                                      | Recovered            | 9                         |
| A02†       | M/2                 | 12                          | 2                                      | Recovered            | 11                        |
| A03‡       | F/2                 | 16                          | 3                                      | Recurrent            | NA                        |
| A04†       | F/2                 | 8                           | 5                                      | Recovered            | 5                         |

\*NA = not applicable.

†Methylprednisolone acetate treatment group.

‡Non-methylprednisolone acetate treatment group.

shedding levels for all four of the cats that were re-infectable; the peak levels of virus shedding were as high as observed during primary infection and the duration was similar (4–7 months). No evidence for reinfection was observed in cats that had been shedding high levels of virus at the time of secondary challenge exposure (Fig 6).

#### **Relationship of age to peak virus shedding during primary infection**

Cats were divided into three age groups: (1) kittens 2–4 months of age at the time of primary infection ( $n = 22$ ), (2) mature cats 2–8 years of age ( $n = 25$ ), and (3) aged cats 8–13 years of age

( $n = 4$ ). The peak level of virus shedding during their primary phase of FECV infection was compared between groups (Fig 7). Kittens shed significantly higher peak levels of virus than cats 2–8 years of age; virus shedding was also higher than for aged cats, but this difference was not significant. Aged cats (8–13 years of age) also tended to shed higher levels than 2–8 year olds, but the difference was also not significant.

#### **Relationship of serum antibody titers and virus shedding status**

Antibodies to FCoV were measured sequentially by the IFA procedure in 16 animals over a period



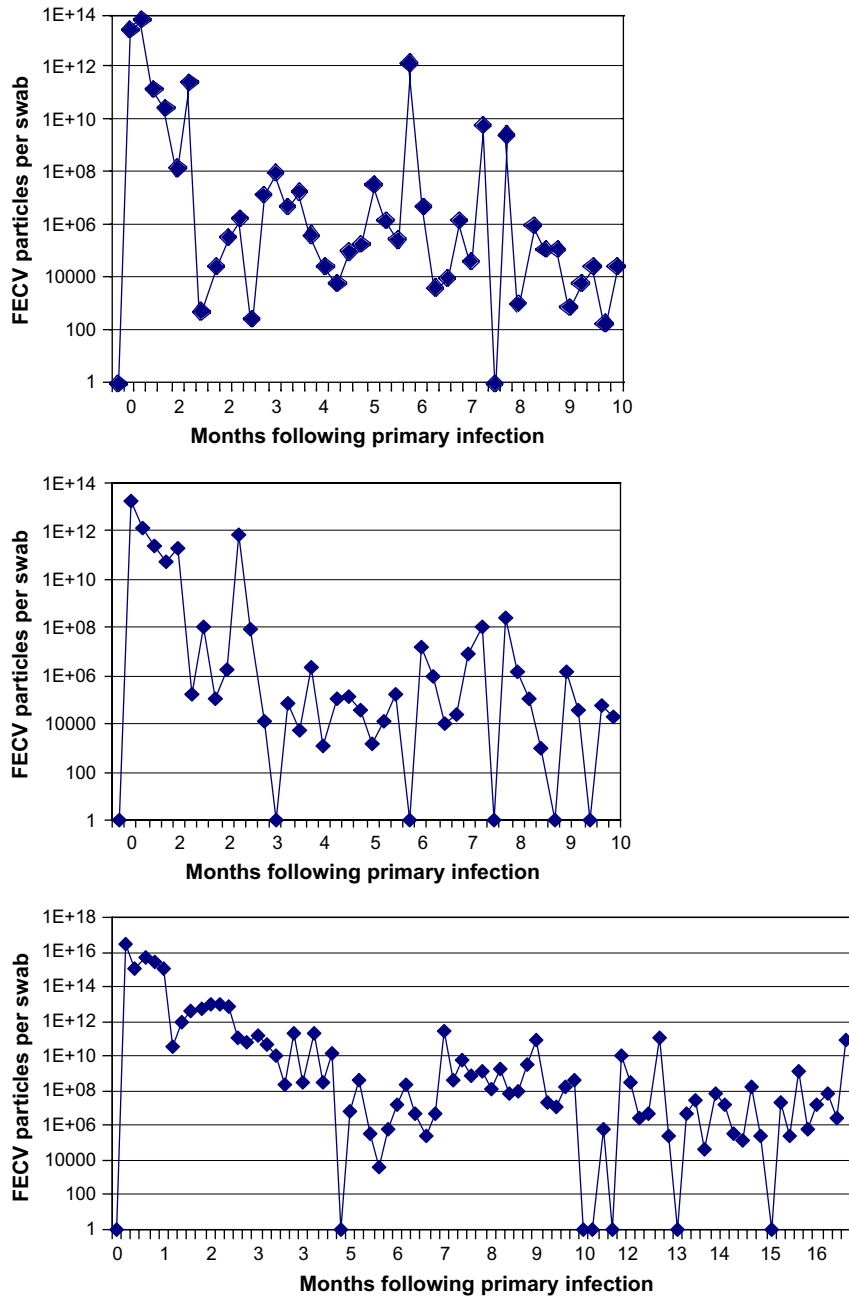


Fig 1. Typical fecal FECV shedding patterns of cats demonstrating a persistent pattern of infection.

of 12–24 months. These cats were randomly selected from among the 33 animals whose infection course had been established. A total of 241 time matched serum/feces samples were analyzed (Fig 8). FCoV antibody titers were significantly ( $P = 0.05$ ) higher among cats that were virus shedders at the time of testing than in the group of cats that were non-shedders. Conversely, cats with titers of 1:25 and lower, as a group, were significantly ( $P = 0.05$ ) more likely to be non-shedders. However, there was considerable overlap in titers

and virus shedding status among individual cats in the two groups; virus shedders and non-shedders were to be found in individuals with the lowest (5–25) and highest (1600) titers.

**Natural transmission to kittens born to project queens**

Twenty-two kittens were born to eight different queens, and data was available for 12 of them for the first 24 weeks of their lives. None of

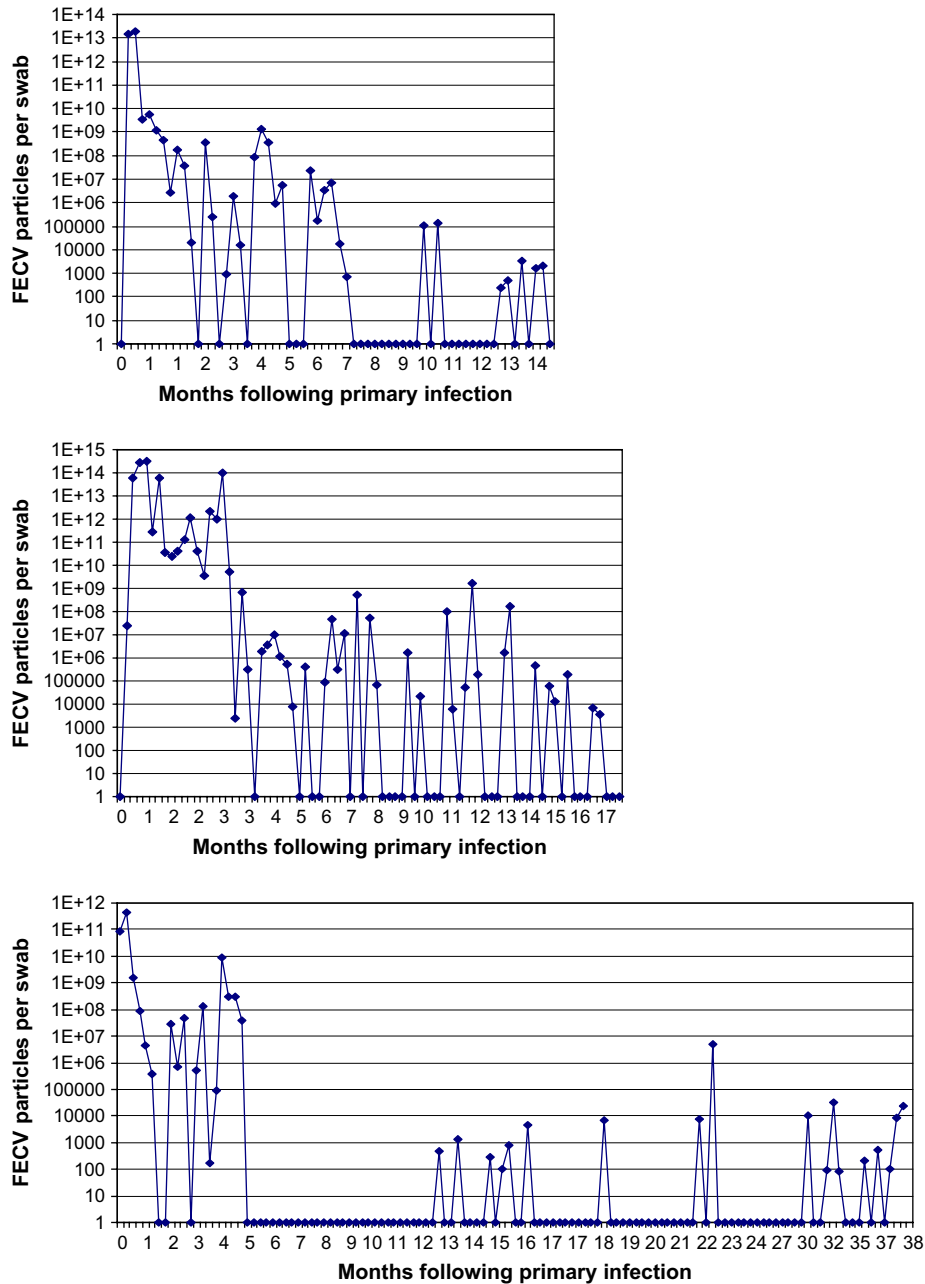


Fig 2. Typical fecal FECV shedding patterns of cats demonstrating an intermittent pattern of infection.

these 12 kittens shed FECV before 9 weeks of age, while all kittens tested at 9–11 weeks of age were shedding as a result of natural exposure (Fig 9). However, the level of virus shedding was relatively low, from  $10^3$  to  $10^8$  particles/swab, and declined to very low or non-detectable levels by 13–15 weeks (Fig 9). All of the kittens were infected with FECV-RM at 10–17 weeks of age (average 13 weeks) regardless of their prior FECV shedding status. The subsequent pattern of fecal virus shedding resembled that observed during primary

FECV exposure in coronavirus naïve cats (Figs 1–3, 9).

#### ***Effects of pregnancy, parturition and lactation on FECV shedding***

Fecal virus shedding was measured for a period of 12 weeks before and 12 weeks after parturition in seven queens and nine litters (Fig 10). There was no significant difference in the levels of FECV shedding as a result of pregnancy, parturition or lactation.

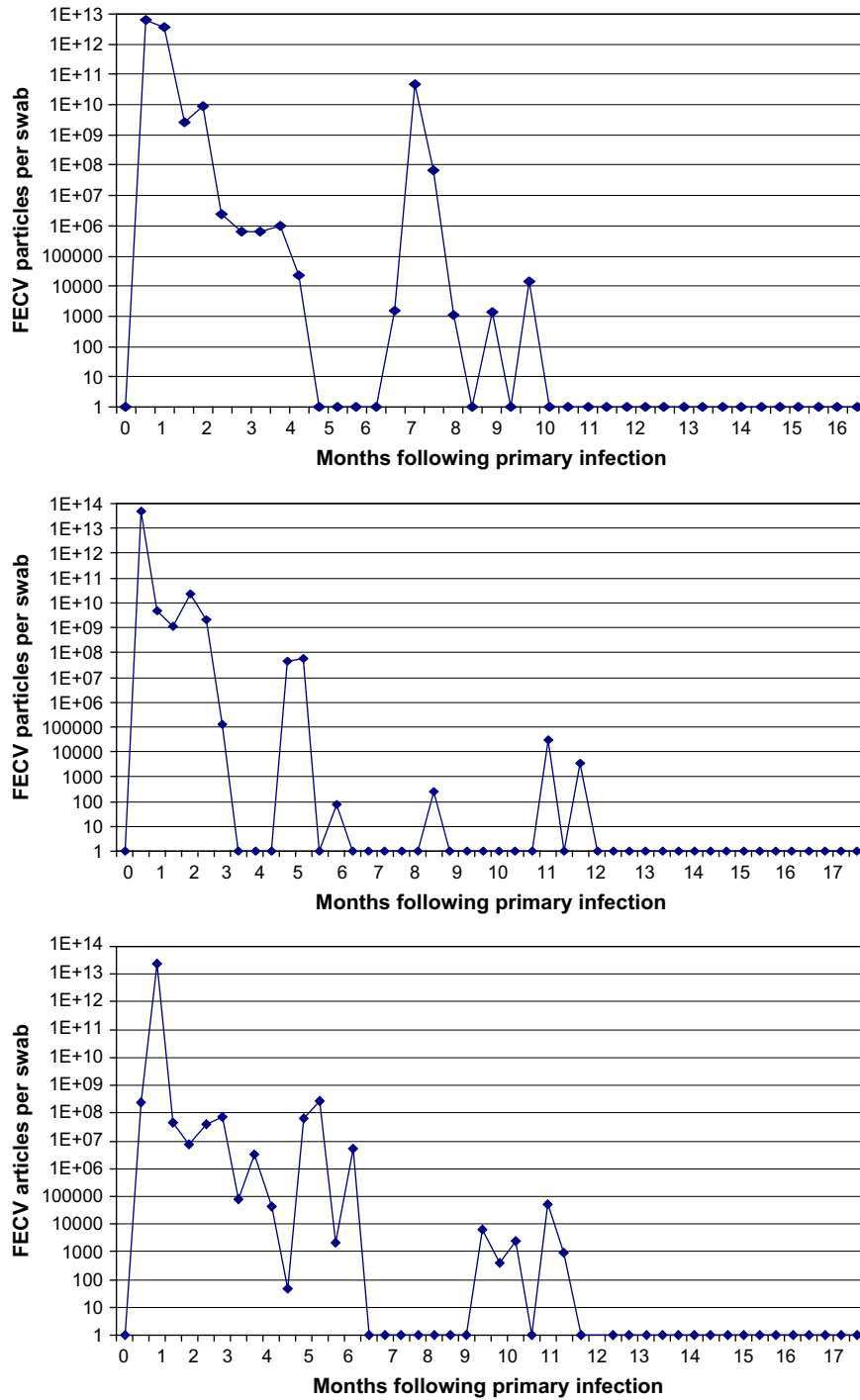


Fig 3. Typical fecal FECV shedding patterns of cats demonstrating a self-limiting (recovery) pattern of infection.

**Effects of methylprednisolone acetate treatment on fecal FECV shedding**

Methylprednisolone acetate (5 mg/kg) was administered twice intramuscularly at a 3-week interval to 10 randomly selected cats from the project; eight cohort cats were given saline (Table 1). There was no statistical change in the

levels of virus shedding post-treatment in cats given methylprednisolone acetate (Fig 11) or saline (data not shown). Furthermore, cats in either group that were shedding at the time of treatment were not induced to shed more virus and cats that were non-shedders did not start shedding (data not shown).



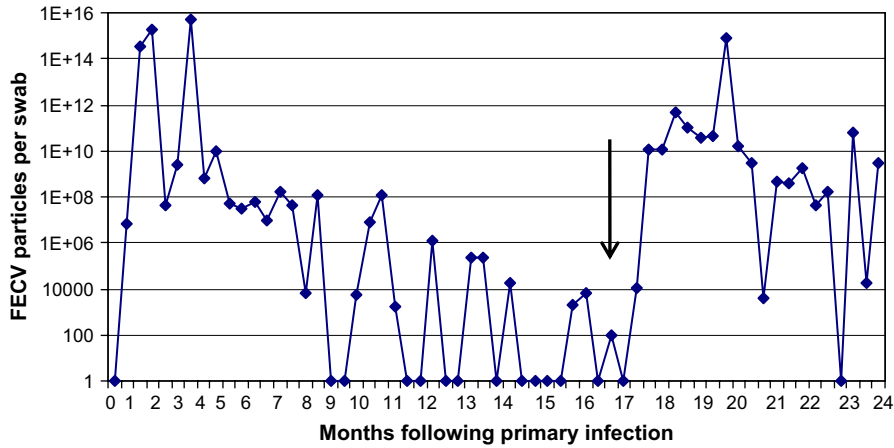


Fig 4. Levels of virus shedding prior to and after reinfection (arrow).

**Discussion**

The present study added to our understanding of the course of FECV infection in domestic cats. There was a distinct primary stage of infection that lasted from 7 to 18 months; the highest level of virus shedding occurred during this stage. This primary stage was resolved in one of three manners: (1) recovery, (2) persistent shedding, and (3) recurrent or intermittent shedding. These findings corroborated earlier studies of naturally occurring FECV infection. In a comprehensive study of 275 purebred cats from six catteries, fecal samples were collected every 1–3 months for a year and virus shedding quantified by RT-PCR (Foley et al 1997b). A large proportion of these cattery cats shed virus at any given time, but most manifested cycles of infection and shedding. Similarly, Harpold et al (1999) found that all adult cats in an Abyssinian

cattery shed virus in their feces at least once during the year and 4/15 cats were shedding greater than 75% of the time. Rohner (1999) reported that FECV shedding dramatically decreased over 2 years in a group of naturally infected cats. Herrewegh et al (1997) studied the persistence and evolution of endemic FECV infection in a closed cat-breeding facility. Viral RNA was detected by RT-PCR in the feces and/or plasma of 36 of 42 cats (86%) tested. Four of five infected cats were still shedding when tested 111 days later. Two cats were then placed in strict isolation and virus shedding was found to last up to 7 months in one animal.

Persistent and recovered infections might be explained by relative differences in the strength of local gut immunity. However, an immunologic explanation for the recurrent pattern of shedding was not so apparent. It would be tempting to blame periods of recurrent shedding on frequent reinfections. Reinfection is a common occurrence

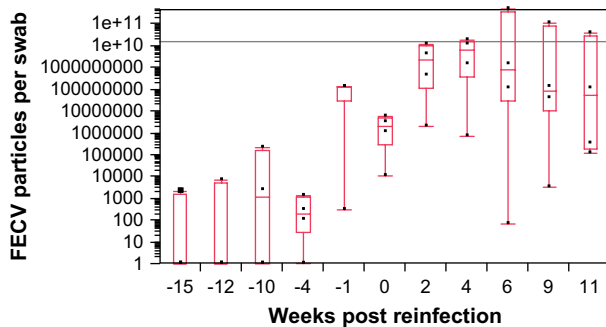


Fig 5. One-way analysis of levels of fecal FECV shedding in a group of four cats that were shedding very low or non-detectable levels of virus prior to infection. Virus levels following reinfection were higher at all time points than they were prior to infection, but because of the small group size, only weeks 3 and 4 were significantly different.

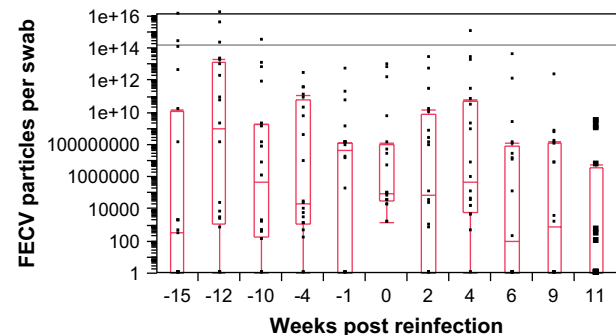


Fig 6. One-way analysis of levels of fecal FECV shedding in a group of 15 cats that were shedding virus at the time of their secondary challenge exposure. There was no significant change in virus shedding following reinfection.

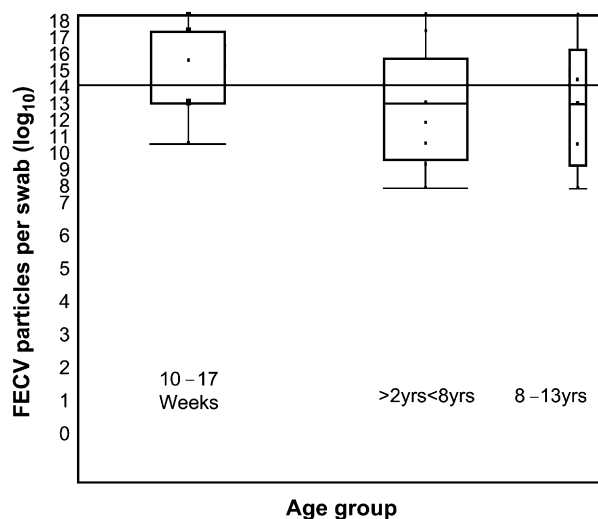


Fig 7. One-way analysis of the mean peak levels of FECV fecal shedding during primary infection in cats infected at 2–4 months of age, >2 < 8 years of age, and >8 years of age.

for many gut pathogens, because local immunity often requires persistence of the organism and does not possess strong memory (Brandtzaeg 2007). However, successful reinfection in four cats resembled a primary infection in magnitude and duration, which was not true for recurrent shedding. It is possible that recurrent shedding was an artifact of the assay procedure. If the assay failed to delineate low level shedding from non-shedding, recurrent and persistent shedders would be basically the same except for amplitude. The alternative possibility was that these periods of reshedding were due to reactivation of a latent or sub-detectable infection. However, this was not supported by studies of natural or artificial stress (see below).

FECV was shed at very high levels following primary infection and the levels were significantly higher in kittens than in adult cats. Rohner (1999) also found that the levels of FECV were

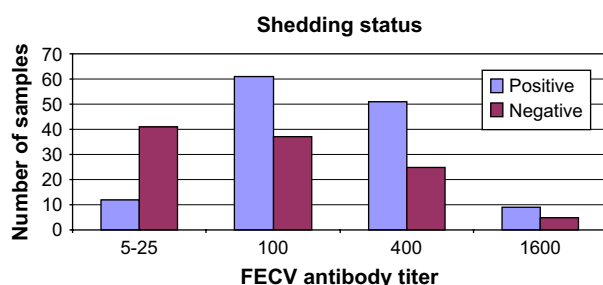


Fig 8. FCoV indirect IFA antibody titers in serum collected from cats over a 12–24 month period. Their fecal FECV shedding status was measured at the same time.

many log<sub>10</sub> higher during early than late infection. Foley et al (1997b) were the first to show higher levels of FECV shedding in kittens than older cats in shelters. These findings have an important collective implication for FIP. FIP is much more common among younger cats (Pedersen 1976, Foley et al 1997a). If kittens are infected before their immune systems are fully matured, levels of FECV replication would be higher, and greater levels of virus replication would favor FECV → FIPV mutations. Relative age-related immunodeficiency could also prevent kittens from containing the FIP mutant virus. This scenario is supported by research with FECV-RM infection in cats that were immunocompromised by long-standing FIV infection (Poland et al 1996). Chronic FIV infected cats shed 10–100 times more FECV than age-matched non-FIV infected cats, just like FECV in kittens, and 2/19 of them developed FIP vs none of the 20 FIV free cohorts. It was concluded that immunosuppression caused by chronic FIV infection enhanced the creation and selection of FIPV mutants by increasing the rate of FECV replication in the bowel, as well as by inhibiting the host's ability to combat the mutant viruses once they occurred.

The study also followed kittens born to FECV infected queens. None of these kittens shed virus prior to 9 weeks of age, while all kittens that were tested between 10 and 15 weeks were positive. These findings were in concordance with those of Foley et al (1997a,b), who were not able to detect virus in feces before 10 weeks of age in cattery kittens. However, Harpold et al (1999) found that kittens in an Abyssinian cattery started shedding virus at 33–78 days (5–11 weeks) of age (mean 9.6 weeks). Gut et al (1999) studied 77 kittens from 12 catteries and found a progressive rise in fecal shedding from around 2–4 weeks onwards, with a peak at 9 weeks of age. Therefore, the period of 9 weeks of age is probably when most kittens are infected, although it may occur at an earlier age under certain conditions. These chronological findings support FCoV control programs that advocate isolation of pregnant queens and early weaning of their kittens (Addie et al 2004). Theoretically, if queens are strictly isolated and their kittens separated at the earliest possible time (<5–6 weeks of age), the kittens will remain, for the most part, free of FECV. The problem with this procedure is the occasional infection of kittens at 2–5 weeks of age (Gut 1999, Harpold et al 1999) as well as the problem of preventing infection after weaning. FECV is easily

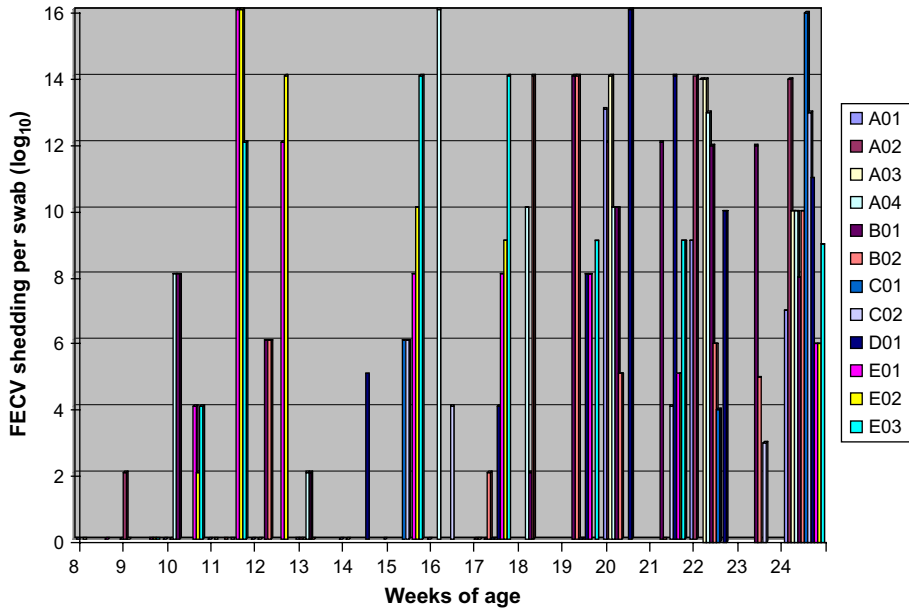


Fig 9. Fecal virus shedding levels in kittens born to project queens. Kittens were infected naturally at 9–10 weeks of age, but this infection appeared transient. Kittens were experimentally infected at 10–17 weeks of age (average 13 weeks).

transmitted from room to room by caregivers even with very good containment facilities and procedures (Pedersen et al 1981a). The ease of fomite transmission and ubiquitous nature of the virus makes it extremely difficult to keep cats free of the virus. However, delaying FECV infection until the kittens are older (>16 weeks), by isolation and early weaning, may have another

depressing effect on FIP regardless of whether or not they are infected later in life. The levels of virus replication might be lower in older kittens, thus decreasing the likelihood of mutants, and the immune system would be more competent in containing any FIPV that might arise.

Kittens born into the study, and infected naturally, demonstrated a peculiar form of infection.

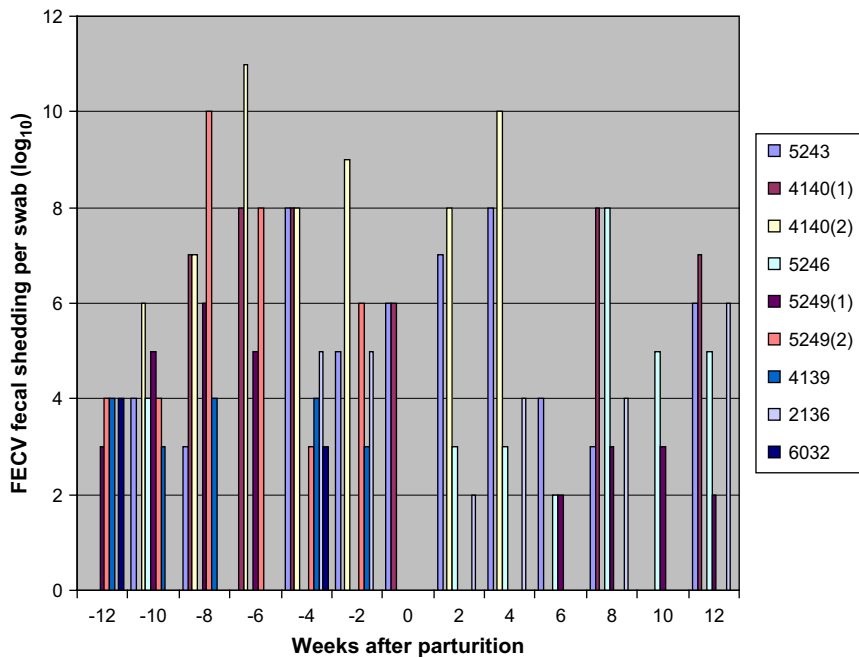
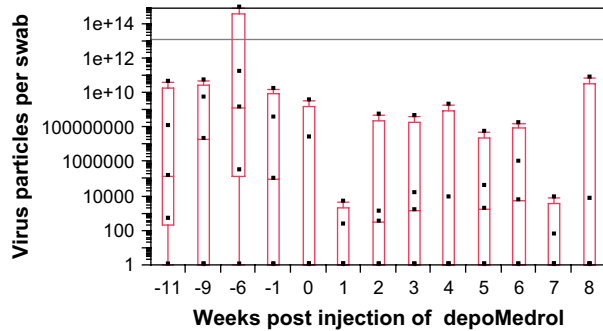


Fig 10. Average levels of FECV fecal shedding before and after parturition in seven queens during nine pregnancies.



**Fig 11.** Levels of fecal FECV in cats that were positive shedders and treated with two injections of methylprednisolone acetate at week 0 and 4. There was no significant difference in levels of virus shed in the feces after treatment.

The levels of virus replication in naturally infected kittens were much lower than in older kittens and cats that had been experimentally infected. The virus shedding also seemed to be much more transient. Unlike cats with longer term infections, which did not respond to super-infection, kittens responded to an experimental challenge exposure just like naïve or recovered cats. This suggests that maternal immunity may have played some role in altering the course of natural infection, although not leading to either strong premonition or adaptive immunity.

The role of stress in reactivating possible latent or subclinical infections, or increasing virus shedding among shedders, was studied in two ways: (1) by studying a natural stress, ie, pregnancy, parturition and lactation, and (2) inducing an artificial stress with corticosteroid treatment. The stress of parturition and lactation is known to activate latent feline herpesvirus infection in about 40% of queens, and this re-excretion of virus is an important route for infection of kittens (Gaskell and Povey 1977). This stress can be mimicked by the administration of corticosteroids (Gaskell and Povey 1977, Hickman et al 1994); a single dose of methylprednisolone acetate is particularly effective (Reubel et al 1993). Methylprednisolone is also a potent activator of latent feline leukemia virus infection (Rojko et al 1982, Pedersen et al 1984a,b) and will abolish age-related resistance to FeLV (Pedersen and Johnson, 1991). It will even reactivate subclinical dermatophyte infections in kittens in the post-recovery phase (Pedersen 1991). Unlike feline herpesvirus, neither parturition/lactation nor methylprednisolone treatment affected FECV shedding in this laboratory setting.

It might be argued that conditions in nature are more severe; however, FECV shedding was also not affected by parturition and lactation in cattery cats (Foley et al 1997b).

There was a significant relationship between serum IFA titers and virus shedding in this study. Cats that were shedding virus tended to have IFA titers of 1:100 and above. Cats that were no longer shedding virus tended to have titers of 1:25 and lower. This confirmed an earlier report by Rohner (1999). However, such a relationship was not noted by Harpold et al (1999) or insinuated by Foley et al (1997b). This discrepancy may involve the manner in which data is viewed. When fecal shedders and non-shedders are looked at as groups, the relationship between higher titers and shedding and lower titers and non-shedding was significant. However, the overlap between titer and shedding was substantial and greatly diminished the value of titers in evaluating shedding status in individual cats. The value of applying group FECV.

Application of this technique to eradication of FECV under experimental conditions was first reported by Hickman et al (1995). A FECV was inadvertently introduced into a very large research cat-breeding colony and not recognized until a few cases of FIP were confirmed over the next couple of years. In order to save valuable blood lines, FECV was eradicated based on serum antibody titers. Cats with high or rising titers were culled and cats with low and decreasing titers were taken out of breeding and maintained in strict isolation. The process of continually selecting cats with low titers eventually yielded a much smaller group of cats that were proven to be free of FECV by following titers in their kittens. If the titers in kittens were strictly of maternal origin, they would become negative by 12–16 weeks. If the kittens were infected, they would fall, and then rise again after 12–16 weeks. Such an eradication regimen requires exceptional quarantine facilities and infection control practices and accurate titer determinations. These are difficult to achieve in most multi-cat environments in the field. Although the use of group titers for eradication may not be useful to many multi-cat households and catteries, group titers may be helpful in looking at the overall coronavirus status in cattery or other multi-cat environments. Groups of cats that tend to have high titers are likely to have a significant proportion of FECV shedders, while the converse would be true for groups of cats that have low and negative titers. Catteries with

many high titer cats are known to have a greater incidence of FIP (Foley et al 1997a,b).

Immunity to FECV infection was not studied per se, but it was possible to infer several things from the present observations. First, there is a definite primary stage of infection that lasts several months, followed by a period when virus shedding either remains persistent at a lower level, becomes intermittent, or ceases. The immunity generated during this primary stage was slow to develop, variable in strength, and tenuous in duration. Reinfection also appeared to mirror primary infection, indicating that immunity is not only tenuous, but that it lacks memory. The finding that immunity is tenuous and reinfection common mirrors the conclusions of Addie et al (2003), who found that FECV recovered cats can be reinfected with the same or different strains of the virus. This is characteristic of gut immunity in general (Brandtzaeg 2007) and to coronavirus immunity in particular (Saif 2004). This pattern of infection and immunity is strongly influenced by environmental factors. FECV infection would be self-limiting if groups of cats were allowed to grow old without constant re-exposure to other infected cats and to new cats (especially kittens). Certain husbandry practices unique to cats may also favor cat-to-cat (ie, litterboxes and litter) and cat-to-human-to-cat (fomite) transmission.

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